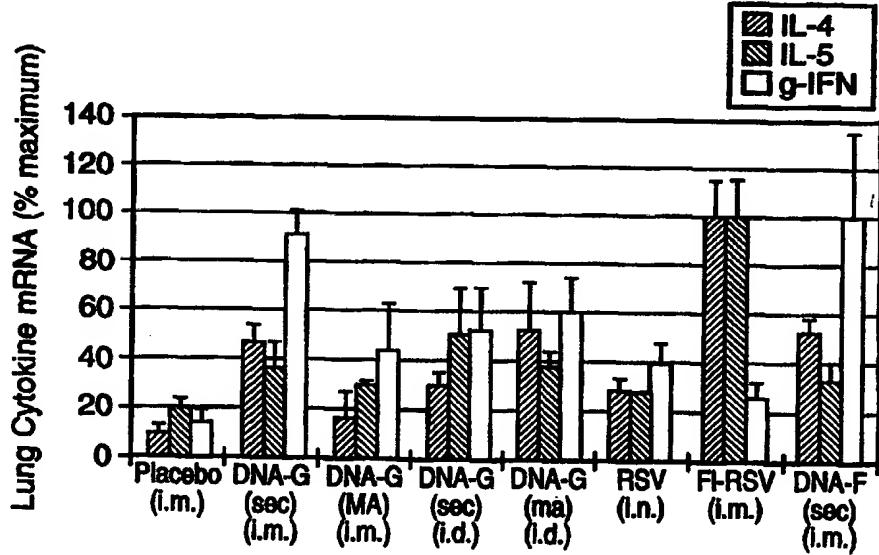




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(54) Title: NUCLEIC ACID VACCINES ENCODING G PROTEIN OF RESPIRATORY SYNCYTIAL VIRUS



(57) Abstract

Non-replicating vectors, such as plasmid vectors, containing a nucleotide sequence coding for a G protein of respiratory syncytial virus (RSV) and a promoter for such sequence, preferably a cytomegalovirus promoter, are described. Such vectors also may contain a further nucleotide sequence located adjacent to the RSV G protein encoding sequence to enhance the immunoprotective ability of the RSV G protein when expressed *in vivo*. Such non-replicating vectors may be used to immunize a host, including a human host, against RSV infection by administration thereto. Such non-replicating vectors also may be used to produce antibodies for detection of RSV infection in a sample.

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TITLE OF INVENTIONNUCLEIC ACID VACCINES ENCODING G PROTEIN OF
RESPIRATORY SYNCYTIAL VIRUS

5

FIELD OF INVENTION

The present invention is related to the field of respiratory syncytial virus (RSV) vaccines and is particularly concerned with vaccines comprising nucleic acid sequences encoding the attachment (G) protein of RSV.

BACKGROUND OF INVENTION

Respiratory syncytial virus (RSV), a negative-strand RNA virus belonging to the *Paramyxoviridae* family of viruses, is the major viral pathogen responsible for bronchiolitis and pneumonia in infants and young children (ref. 1 - Throughout this application, various references are referred to in parenthesis to more fully describe the state of the art to which this invention pertains. Full bibliographic information for each citation is found at the end of the specification, immediately preceding the claims. The disclosures of these references are hereby incorporated by reference into the present disclosure). Acute respiratory tract infections caused by RSV result in approximately 90,000 hospitalizations and 4,500 deaths per year in the United States (ref. 2). Medical care costs due to RSV infection are greater than \$340 M annually in the United States alone (ref. 3). There is currently no licensed vaccine against RSV. The main approaches for developing an RSV vaccine have included inactivated virus, live-attenuated viruses and subunit vaccines.

A protective immune response against RSV is thought to require the induction of neutralizing antibodies against the surface fusion (F) and attachment (G) glycoproteins (ref. 4). In addition, cytotoxic T lymphocytes (CTL) responses are involved in viral clearance. The F protein is conserved amongst the RSV A

and B subgroups.

The G protein (33 kDa) of RSV is heavily O-glycosylated giving rise to a glycoprotein of apparent molecular weight of 90 kDa (ref. 5). Two broad subtypes 5 of RS virus have been defined: A and B (ref. 6). The major antigenic differences between these subtypes are found in the G glycoprotein (refs. 3, 7).

The use of RSV proteins as vaccines may have obstacles. Parenterally administered vaccine candidates 10 have so far proven to be poorly immunogenic with regard to the induction of neutralizing antibodies in seronegative chimpanzees. The serum antibody response induced by these antigens may be further diminished in the presence of passively acquired antibodies, such as 15 the transplacentally acquired maternal antibodies which most young infants possess. A subunit vaccine candidate for RSV consisting of purified fusion (F) glycoprotein from RSV infected cell cultures and purified by immunoaffinity or ion-exchange chromatography has been 20 described (ref. 8). Parenteral immunization of seronegative or seropositive chimpanzees with this preparation was performed and three doses of 50 µg were required in seronegative animals to induce an RSV serum neutralizing titre of approximately 1:50. Upon 25 subsequent challenge of these animals with wild-type RSV, no effect of immunization on virus shedding or clinical disease could be detected in the upper respiratory tract. The effect of immunization with this vaccine on virus shedding in the lower respiratory tract 30 was not investigated, although this is the site where the serum antibody induced by parenteral immunization may be expected to have its greatest effect. Safety and immunogenicity studies have been performed in a small number of seropositive individuals. The vaccine was 35 found to be safe in seropositive children and in three

seronegative children (all > 2.4 years of age). The effects of immunization on lower respiratory tract disease could not be determined because of the small number of children immunized. One immunizing dose in 5 seropositive children induced a 4-fold increase in virus neutralizing antibody titres in 40 to 60% of the vaccinees. Thus, insufficient information is available from these small studies to evaluate the efficacy of this vaccine against RSV-induced disease. A further 10 problem facing subunit RSV vaccines is the possibility that inoculation of seronegative subjects with immunogenic preparations might result in disease enhancement. In the 1960's, vaccination of infants with a formalin-inactivated RSV preparation (FI-RSV) resulted 15 in enhanced lung disease upon subsequent exposure to live virus, also referred to as immunopotentiation (refs. 9, 10). These vaccinees developed strong serological responses, but were not protected against infection and some developed severe, occasionally fatal 20 respiratory tract disease upon natural infection. Although precise mechanisms remain unknown, it has been suggested that this form of immune enhancement might reflect either structural alterations of RSV antigens (ref. 11), residual serum and/or cellular contaminants 25 (ref. 12), a specific property of the viral attachment (G) protein (refs. 13,14) or an imbalanced cell-mediated immune response (refs. 13,15). It has been demonstrated that the FI-RSV vaccine induced a TH2-type immune response in mice whereas immunization with live RSV, 30 which does not cause immunopotentiation, elicits a TH1 response (ref.15).

In some studies, the immune response to immunization with a synthetic RSV FG fusion protein resulted in disease enhancement in rodents resembling 35 that induced by a formalin-inactivated RSV vaccine.

Immunization of mice with a recombinant vaccinia virus expressing the RSV G protein resulted in G-specific T cell responses in the lungs which are exclusively recruited from the CD4+T cell sublineage and are 5 strongly Th2-biased. G-specific T cells induce lung haemorrhage, pulmonary neutrophil recruitment (shock lung), intense pulmonary eosinophilia, and sometimes death in the adoptively transferred murine recipients (ref. 14). The association of immunization with disease 10 enhancement using certain vaccine preparations including non-replicating antigens suggests caution in their use as vaccines in seronegative humans.

Live attenuated vaccines against disease caused by RSV may be promising for two main reasons. Firstly, 15 infection by a live vaccine virus induces a balanced immune response comprising mucosal and serum antibodies and cytotoxic T-lymphocytes. Secondly, infection of infants with live attenuated vaccine candidates or naturally acquired wild-type virus is not associated 20 with enhanced disease upon subsequent natural reinfection. It will be challenging to produce live attenuated vaccines that are immunogenic for younger infants who possess maternal virus-neutralizing antibodies and yet are attenuated for seronegative 25 infants greater than or equal to 6 months of age. Attenuated live virus vaccines also have the risks of residual virulence and genetic instability.

Injection of plasmid DNA containing sequences encoding a foreign protein has been shown to result in 30 expression of the foreign protein and the induction of antibody and cytotoxic T-lymphocyte (CTL) responses to the antigen in a number of studies (see, for example, refs. 16, 17, 18). The use of plasmid DNA inoculation to express viral proteins for the purpose of 35 immunization may offer several advantages over the

strategies summarized above. Firstly, DNA encoding a viral antigen can be introduced in the presence of antibody to the virus itself, without loss of potency due to neutralization of virus by the antibodies.

5 Secondly, the antigen expressed *in vivo* should exhibit a native conformation and the appropriate glycosylation. Therefore, the antigen should induce an antibody response similar to that induced by the antigen present in the wild-type virus infection. In contrast, some

10 processes used in purification of proteins can induce conformational changes which may result in the loss of immunogenicity of protective epitopes and possibly immunopotentiation. Thirdly, the expression of proteins from injected plasmid DNAs can be detected *in vivo* for a

15 considerably longer period of time than that in virus-infected cells, and this has the theoretical advantage of prolonged cytotoxic T-cell induction and enhanced antibody responses. Fourthly, *in vivo* expression of antigen may provide protection without the need for an

20 extrinsic adjuvant.

The ability to immunize against disease caused by RSV by administration of a DNA molecule encoding an RSV G protein was unknown before the present invention. In particular, the efficacy of immunization against RSV

25 induced disease using a gene encoding a secreted form of the RSV G protein was unknown. Infection with RSV leads to serious disease. It would be useful and desirable to provide isolated genes encoding RSV G protein and non-replicating vectors, including plasmid vectors, for *in*

30 *vivo* administration and for use in immunogenic preparations, including vaccines, for protection against disease caused by RSV and for the generation of diagnostic reagents and kits. In particular, it would be desirable to provide vaccines that are immunogenic

35 and protective in humans, including seronegative

infants, that do not cause disease enhancement (immunopotentiation).

SUMMARY OF INVENTION

The present invention relates to a method of
5 immunizing a host against disease caused by respiratory syncytial virus, to non-replicating vectors containing nucleic acid molecules used in immunogenic compositions for such purpose, and to diagnostic procedures utilizing the vectors and nucleic acid molecules. In particular,
10 the present invention is directed towards the provision of nucleic acid vaccines encoding the G protein of respiratory syncytial virus.

In accordance with one aspect of the invention, there is provided an immunogenic composition for *in vivo* 15 administration to a host for the generation in the host of protective antibodies to respiratory syncytial virus (RSV) G protein, comprising a non-replicating vector comprising:

a first nucleotide sequence encoding a RSV G 20 protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and

25 a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein *in vivo* from said vector in the host, and

a pharmaceutically-acceptable carrier therefor.

30 The first nucleotide sequence may be that which encodes a full-length RSV G protein. The first nucleotide sequence may comprise the nucleotide sequence shown in Figure 2 (SEQ. ID No: 1) or encode a full length RSV G protein having the amino acid sequence 35 shown in Figure 2 (SEQ. ID no: 2).

Alternatively, the first nucleotide sequence may be that which encodes an RSV G protein from which the transmembrane coding sequence and sequences upstream thereof are absent. The first nucleotide sequence 5 encoding the truncated RSV G protein may comprise the nucleotide sequence shown in Figure 3 (SEQ. ID no: 3) or may comprise a nucleotide sequence encoding the truncated RSV G protein having the amino acid sequence shown in Figure 3 (SEQ ID no: 4). The lack of 10 expression of the transmembrane region results in a secreted form of the RSV G protein.

The non-replicating vector may further comprise a heterologous signal peptide encoding nucleotide sequence immediately upstream of the 5'-terminus of the first 15 nucleotide sequence. The signal peptide encoding sequence may encode the signal peptide of human tissue plasminogen activator.

The promoter sequence may be an immediate early cytomegalovirus (CMV) promoter. The second nucleotide 20 sequence may comprise the human cytomegalovirus Intron A.

The non-replicating vector generally is a plasmid vector. Plasmid vectors encoding the G protein and included in the immunogenic composition provided by this 25 aspect of the invention may specifically be pXL5 or pXL6, constructed and having their characterizing elements, as seen in Figures 4 or 5, respectively.

In accordance with a further aspect of the present invention, there is provided a method of immunizing a 30 host against disease caused by infection with respiratory syncytial virus (RSV), which comprises administering to the host an effective amount of a non-replicating vector comprising:

a first nucleotide sequence encoding an RSV G 35 protein or a RSV G protein fragment that generates

antibodies that specifically react with RSV G protein,
a promoter sequence operatively coupled to said
first nucleotide sequence for expression of said RSV G
protein in the host, and

- 5 a second nucleotide sequence located between said
first nucleotide sequence and said promoter sequence to
increase expression of said RSV G protein *in vivo* from
said vector in the host.

The immunization method may be effected to induce a
10 balanced Th1/Th2 immune response.

The present invention also includes a novel method
of using a gene encoding respiratory syncytial virus
(RSV) G protein or a RSV G protein fragment that
generates antibodies that specifically react with RSV G
15 protein, to protect a host against disease caused by
infection with respiratory syncytial virus, which
comprises:

isolating the gene;
operatively linking the gene to at least one
20 control sequence to produce a non-replicating vector,
said control sequence directing expression of the RSV G
protein when said vector is introduced into a host to
produce an immune response to the RSV G protein, and
introducing the vector into the host.

- 25 The procedure provided in accordance with this aspect of
the invention may further include the step of:

operatively linking the gene to an immunoprotection
enhancing sequence to produce an enhanced
immunoprotection by the RSV G protein in the host,
30 preferably by introducing the immunoprotection enhancing
sequence between the control sequence and the gene,
including introducing immunostimulatory CpG sequences in
the vector.

In addition, the present invention includes a
35 method of producing a vaccine for protection of a host

against disease caused by infection with respiratory syncytial virus (RSV), which comprises:

isolating a first nucleotide sequence encoding an RSV G protein or a RSV G protein fragment that generates 5 antibodies that specifically react with RSV G protein,

operatively linking the first nucleotide sequence to at least one control sequence to produce a non-replicating vector, the control sequence directing expression of the RSV G protein when introduced into a 10 host to produce an immune response to the RSV G protein when expressed *in vivo* from the vector in a host,

operatively linking the first nucleotide sequence to a second nucleotide sequence to increase expression 15 of the RSV G protein *in vivo* from the vector in a host, and

formulating the vector as a vaccine for *in vivo* administration.

The vector may be a plasmid vector selected from pXL5 and pXL6. The invention further includes a vaccine 20 for administration to a host, including a human host, produced by this method.

As noted previously, the vectors provided herein are useful in diagnostic applications. In a further aspect of the invention, therefore, there is provided a 25 method of determining the presence of a respiratory syncytial virus (RSV) G protein in a sample, comprising the steps of:

(a) immunizing a host with a non-replicating 30 vector to produce antibodies specific for the RSV G protein, the non-replicating vector comprising a first nucleotide sequence encoding an RSV G protein or an RSV G protein fragment that generates antibodies that specifically react with RSV G protein, a promoter sequence operatively coupled to 35 the first nucleotide sequence for expression of the

RSV G protein in the host and a second nucleotide sequence located between the first nucleotide sequence and the promoter sequence to increase expression of the RSV G protein *in vivo* from the vector in the host;

- 5 (b) isolating the RSV G protein-specific antibodies;
- 10 (c) contacting the sample with the isolated antibodies to produce complexes comprising any RSV G protein present in the sample and the RSV G protein-specific antibodies; and
- 15 (d) determining production of the complexes.

The non-replicating vector employed to elicit the antibodies may be a plasmid vector pXL5 or pXL6.

- 15 The invention also includes a diagnostic kit for detecting the presence of a respiratory syncytial virus (RSV) G protein in a sample, comprising:
- 20 (a) a non-replicating vector capable of generating antibodies specific for the RSV G protein when administered to a host, said non-replicating vector comprises a first nucleotide sequence encoding an RSV G protein or an RSV G protein fragment that generates antibodies that specifically react with RSV G protein, a promoter sequence operatively coupled to the first nucleotide sequence for expression of the RSV G protein in a host, and a second nucleotide sequence located between the first nucleotide sequence and the promoter sequence to increase expression of the RSV G protein *in vivo* from the vector in the host;
- 25 (b) isolation means to isolate the RSV G protein specific antibodies;
- 30 (c) contacting means to contact the isolated RSV G protein-specific antibodies with the sample to produce a complex comprising any RSV G protein
- 35

present in the sample and RSV G protein specific antibodies; and

(d) identifying means to determine production of the complex.

5 The present invention further is directed to a method for producing antibodies specific for a G protein of a respiratory syncytial virus (RSV) comprising:

10 (a) immunizing a host with an effective amount of a non-replicating vector to produce RSV G-specific antibodies, said non-replicating vector comprising:

15 a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and

20 a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein *in vivo* from said vector in the host; and

(b) isolating the RSV G specific antibodies from the host.

25 The present invention is also directed to a method for producing monoclonal antibodies specific for a G protein of respiratory syncytial virus (RSV), comprising the steps of:

30 (a) constructing a vector comprising a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein, a promoter sequence operatively coupled to the first nucleotide sequence for expression of the RSV G protein in the host and a second nucleotide

sequence located between the first nucleotide sequence and the promoter sequence to increase expression of the RSV G protein when *in vivo* from the vector in a host;

- 5 (b) administering the vector to at least one mouse to produce at least one immunized mouse;
- (c) removing B-lymphocytes from the at least one immunized mouse;
- 10 (d) fusing the B-lymphocytes from the at least one immunized mouse with myeloma cells, thereby producing hybridomas;
- (e) cloning the hybridomas;
- (f) selecting clones which produce anti-RSV G protein antibody;
- 15 (g) culturing the anti-RSV G protein antibody-producing clones; and
- (h) isolating anti-RSV G protein monoclonal antibodies.

Such monoclonal antibodies may be used to purify RSV G protein from virus.

In this application, the term "RSV G protein" is used to define a full-length RSV G protein, such proteins having variations in their amino acid sequences including those naturally occurring in various strains 25 of RSV, a secreted form of RSV G protein lacking a transmembrane region, as well as functional analogs of the RSV G protein. In this application, a first protein is a "functional analog" of a second protein if the first protein is immunologically related to and/or has 30 the same function as the second protein. The functional analog may be, for example, an immunologically-active fragment of the protein or an immunologically-active substitution, addition or deletion mutant thereof.

BRIEF DESCRIPTION OF THE FIGURES

The present invention will be further understood from the following General Description and Examples with reference to the Figures of the accompanying drawings, 5 in which:

Figure 1 illustrates a restriction map of the gene encoding a G protein of respiratory syncytial virus (RSV);

10 Figure 2 illustrates the nucleotide sequence of a gene encoding a membrane bound form of the G protein of respiratory syncytial virus (SEQ ID No: 1) as well as the amino acid sequence of the RSV G protein encoded thereby (SEQ ID No: 2);

15 Figure 3 illustrates the nucleotide sequence of a gene encoding the secreted form of the RSV G protein lacking the transmembrane domain (SEQ ID No: 3) as well as the amino acid sequence of a truncated RSV G protein lacking the transmembrane domain encoded thereby (SEQ ID No: 4);

20 Figure 4 shows the construction of plasmid pXL5 containing a gene encoding a full-length membrane attached form of the RSV G protein and containing the CMV Intron A sequence;

25 Figure 5 shows the construction of plasmid pXL6 containing a gene encoding a secreted form of the RSV G protein lacking the transmembrane domain and containing the CMV Intron A sequence as well as a nucleotide sequence encoding a signal peptide of the human tissue plasminogen activator (TPA);

30 Figure 6 shows the nucleotide sequence for the plasmid VR-1012 (SEQ ID No. 5);

Figure 7 shows the nucleotide sequence for the 5' untranslated region and the signal peptide of the human tissue plasminogen activator (TPA) (SEQ. ID no: 6) and

35 Figure 8 shows the lung cytokine expression profile

in DNA immunized mice after RSV challenge.

GENERAL DESCRIPTION OF INVENTION

As described above, the present invention relates generally to polynucleotide, including DNA, immunization 5 to obtain protection against infection by respiratory syncytial virus (RSV) and to diagnostic procedures using particular non-replicating vectors. In the present invention, several recombinant plasmid vectors were constructed to contain a nucleotide sequence encoding an 10 RSV G protein.

The nucleotide sequence of the full length RSV G gene is shown in Figure 2 (SEQ ID No: 1). Certain constructs provided herein include the nucleotide sequence encoding the full-length RSV G (SEQ ID No: 2) 15 protein while others include an RSV G gene modified by deletion of the transmembrane coding sequence and nucleotides upstream thereof (see Figure 3, SEQ ID No: 3), to produce a secreted or truncated RSV G protein lacking the transmembrane domain (SEQ ID No. 4).

20 The nucleotide sequence encoding the RSV G protein is operatively coupled to a promoter sequence for expression of the encoded RSV G protein *in vivo*. The promoter sequence may be the human immediately early cytomegalovirus (CMV) promoter. This promoter is 25 described in ref. 19. Any other convenient promoter may be used, including constitutive promoters, such as, the Rous Sarcoma Virus LTRs, and inducible promoters, such as the metallothionein promoter, and tissue specific promoters.

30 The non-replicating vectors provided herein, when administered to an animal in the form of an immunogenic composition with a pharmaceutically-acceptable carrier, effect *in vivo* RSV G protein expression, as demonstrated by an antibody response in the animal to which it is 35 administered. Such antibodies may be used herein in the

detection of RSV protein in a sample, as described in more detail below. The administration of the non-replicating vectors, specifically plasmids pXL5 and pXL6, produced anti-G antibodies, virus neutralizing 5 antibodies, a balanced Th1/Th2 response in the lungs post viral challenge and conferred protection in mice against live RSV infection, as seen from the Examples below.

The recombinant vector also may include a second 10 nucleotide sequence located adjacent the RSV G protein encoding nucleotide sequence to enhance the immunoprotective ability of the RSV G protein when expressed *in vivo* in a host. Such enhancement may be provided by increased *in vivo* expression, for example, 15 by increased mRNA stability, enhanced transcription and/or translation. This additional sequence generally is located between the promoter sequence and the RSV G protein-encoding sequence. This enhancement sequence may comprise the immediate early cytomegalovirus Intron 20 A sequence.

The non-replicating vector provided herein may also comprise an additional nucleotide sequence encoding a further antigen from RSV, an antigen from at least one other pathogen or at least one immunomodulating agent, 25 such as a cytokine. Such vector may contain the additional nucleotide sequence in a chimeric or a bicistronic structure. Alternatively, vectors containing the additional nucleotide sequence may be separately constructed and coadministered to a host, 30 along with the non-replicating vectors provided herein.

The non-replicating vector may further comprise a nucleotide sequence encoding a heterologous viral or eukaryotic signal peptide, such as the human tissue plasminogen activator (TPA) signal peptide, in place of 35 the endogenous signal peptide for the truncated RSV G

protein. Such nucleotide sequence may be located immediately upstream of the RSV G encoding sequence in the vector.

The immunogenicity of the non-replicating DNA
5 vectors may be enhanced by inserting immunostimulatory CpG sequences in the vector.

It is clearly apparent to one skilled in the art, that the various embodiments of the present invention have many applications in the fields of vaccination,
10 diagnosis and treatment of RSV infections. A further non-limiting discussion of such uses is further presented below.

1. Vaccine Preparation and Use

Immunogenic compositions, suitable to be used as
15 vaccines, may be prepared from the RSV G genes and vectors as disclosed herein. The vaccine elicits an immune response in an animal which includes the production of anti-RSV G antibodies. Immunogenic compositions, including vaccines, containing the nucleic acid
20 may be prepared as injectables, in physiologically-acceptable liquid solutions or emulsions for polynucleotide administration. The nucleic acid may be associated with liposomes, such as lecithin liposomes or other liposomes known in the art, as a nucleic acid
25 liposome (for example, as described in WO 9324640, ref. 20) or the nucleic acid may be associated with an adjuvant, as described in more detail below. Liposomes comprising cationic lipids interact spontaneously and rapidly with polyanions, such as DNA and RNA, resulting
30 in liposome/nucleic acid complexes that capture up to 100% of the polynucleotide. In addition, the polycationic complexes fuse with cell membranes, resulting in an intracellular delivery of polynucleotide that bypasses the degradative enzymes of the lysosomal
35 compartment. Published PCT application WO 94/27435

describes compositions for genetic immunization comprising cationic lipids and polynucleotides. Agents which assist in the cellular uptake of nucleic acid, such as calcium ions, viral proteins and other 5 transfection facilitating agents, may advantageously be used.

Polynucleotide immunogenic preparations may also be formulated as microcapsules, including biodegradable time-release particles. Thus, U.S. Patent 5,151,264 10 describes a particulate carrier of a phospholipid/glycolipid/polysaccharide nature that has been termed Bio Vecteurs Supra Moléculaires (BVSM). The particulate carriers are intended to transport a variety 15 of molecules having biological activity in one of the layers thereof.

U.S. Patent 5,075,109 describes encapsulation of the antigens trinitrophenylated keyhole limpet hemocyanin and staphylococcal enterotoxin B in 50:50 poly (DL-lactideco-glycolide). Other polymers for 20 encapsulation are suggested, such as poly(glycolide), poly(DL-lactide-co-glycolide), copolyoxalates, polycaprolactone, poly(lactide-co-caprolactone), poly(esteramides), polyorthoesters and poly(8-hydroxybutyric acid), and polyanhydrides.

Published PCT application WO 91/06282 describes a 25 delivery vehicle comprising a plurality of bioadhesive microspheres and antigens. The microspheres being of starch, gelatin, dextran, collagen or albumin. This delivery vehicle is particularly intended for the uptake 30 of vaccine across the nasal mucosae. The delivery vehicle may additionally contain an absorption enhancer.

The RSV G gene containing non-replicating vectors may be mixed with pharmaceutically acceptable excipients which are compatible therewith. Such excipients may 35 include, water, saline, dextrose, glycerol, ethanol, and

combinations thereof. The immunogenic compositions and vaccines may further contain auxiliary substances, such as wetting or emulsifying agents, pH buffering agents, or adjuvants to enhance the effectiveness thereof.

5 Immunogenic compositions and vaccines may be administered parenterally, by injection subcutaneously, intravenously, intradermally or intramuscularly, possibly following pretreatment of the injection site with a local anesthetic. Alternatively, the immunogenic

10 compositions formed according to the present invention, may be formulated and delivered in a manner to evoke an immune response at mucosal surfaces. Thus, the immunogenic composition may be administered to mucosal surfaces by, for example, the nasal or oral

15 (intragastric) routes. Alternatively, other modes of administration including suppositories and oral formulations may be desirable. For suppositories, binders and carriers may include, for example, polyalkylene glycols or triglycerides. Oral

20 formulations may include normally employed incipients, such as, for example, pharmaceutical grades of saccharine, cellulose and magnesium carbonate.

The immunogenic preparations and vaccines are administered in a manner compatible with the dosage

25 formulation, and in such amount as will be therapeutically effective, protective and immunogenic. The quantity to be administered depends on the subject to be treated, including, for example, the capacity of the individual's immune system to synthesize the RSV G

30 protein and antibodies thereto, and if needed, to produce a cell-mediated immune response. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner. However, suitable dosage ranges are readily determinable by one

35 skilled in the art and may be of the order of about 1 µg

to about 2 mg of the RSV G gene-containing vectors. Suitable regimes for initial administration and booster doses are also variable, but may include an initial administration followed by subsequent administrations.

- 5 The dosage may also depend on the route of administration and will vary according to the size of the host. A vaccine which protects against only one pathogen is a monovalent vaccine. Vaccines which contain antigenic material of several pathogens are
10 combined vaccines and also belong to the present invention. Such combined vaccines contain, for example, material from various pathogens or from various strains of the same pathogen, or from combinations of various pathogens.
- 15 Immunogenicity can be significantly improved if the vectors are co-administered with adjuvants, commonly used as 0.05 to 0.1 percent solution in phosphate-buffered saline. Adjuvants enhance the immunogenicity of an antigen but are not necessarily immunogenic
20 themselves. Adjuvants may act by retaining the antigen locally near the site of administration to produce a depot effect facilitating a slow, sustained release of antigen to cells of the immune system. Adjuvants can also attract cells of the immune system to an antigen
25 depot and stimulate such cells to elicit immune responses.

Immunostimulatory agents or adjuvants have been used for many years to improve the host immune responses to, for example, vaccines. Thus, adjuvants have been
30 identified that enhance the immune response to antigens. Some of these adjuvants are toxic, however, and can cause undesirable side-effects, making them unsuitable for use in humans and many animals. Indeed, only aluminum hydroxide and aluminum phosphate (collectively
35 commonly referred to as alum) are routinely used as

adjuvants in human and veterinary vaccines.

A wide range of extrinsic adjuvants and other immunomodulating material can provoke potent immune responses to antigens. These include saponins complexed 5 to membrane protein antigens to produce immune stimulating complexes (ISCOMS), pluronic polymers with mineral oil, killed mycobacteria in mineral oil, Freund's complete adjuvant, bacterial products, such as muramyl dipeptide (MDP) and lipopolysaccharide (LPS), as 10 well as monophoryl lipid A, QS 21 and polyphosphazene.

In particular embodiments of the present invention, the non-replicating vector comprising a first nucleotide sequence encoding an G protein of RSV may be delivered in conjunction with a targeting molecule to target the 15 vector to selected cells including cells of the immune system.

The immunogenicity of the non-replicating vector may be enhanced by coadministering plasmid DNA vectors expressing cytokines or chemokines or by coexpressing 20 such molecules in a bis-cistronic or fusion construct.

The non-replicating vector may be delivered to the host by a variety of procedures, for example, Tang et al. (ref. 21) disclosed that introduction of gold microprojectiles coated with DNA encoding bovine growth 25 hormone (BGH) into the skin of mice resulted in production of anti-BGH antibodies in the mice, while Furth et al. (ref. 22) showed that a jet injector could be used to transfect skin, muscle, fat and mammary tissues of living animals.

30 2. Immunoassays

The RSV G genes and vectors of the present invention are useful as immunogens for the generation of anti-G antibodies for use in immunoassays, including enzyme-linked immunosorbent assays (ELISA), RIAs and 35 other non-enzyme linked antibody binding assays or

procedures known in the art. In ELISA assays, the non-replicating vector first is administered to a host to generate antibodies specific to the RSV G protein. These RSV G-specific antibodies are immobilized onto a selected surface, for example, a surface capable of binding the antibodies, such as the wells of a polystyrene microtiter plate. After washing to remove unadsorbed antibodies, a non-specific protein, such as a solution of bovine serum albumin (BSA) that is known to be antigenically neutral with regard to the test sample, may be bound to the selected surface. This allows for blocking of non-specific adsorption sites on the immobilizing surface and thus reduces the background caused by nonspecific bindings of antisera onto the surface.

The immobilizing surface is then contacted with a sample, such as clinical or biological materials, to be tested in a manner conducive to immune complex (antigen/antibody) formation. This procedure may include diluting the sample with diluents, such as solutions of BSA, bovine gamma globulin (BGG) and/or phosphate buffered saline (PBS)/Tween. The sample is then allowed to incubate for from about 2 to 4 hours, at temperatures such as of the order of about 20° to 37°C. Following incubation, the sample-contacted surface is washed to remove non-immunocomplexed material. The washing procedure may include washing with a solution, such as PBS/Tween or a borate buffer. Following formation of specific immunocomplexes between the test sample and the bound RSV G specific antibodies, and subsequent washing, the occurrence, and even amount, of immunocomplex formation may be determined.

BIOLOGICAL MATERIALS

Certain plasmids that contain the gene encoding the RSV G protein and referred to herein have been deposited

with the American Type Culture Collection (ATCC) located at 12301 Parklawn Drive, Rockville, Maryland, 20852, U.S.A., pursuant to the Budapest Treaty and prior to the filing of this application.

5 Samples of the deposited plasmids will become available to the public upon grant of a patent based upon this United States patent application and all restrictions on access to the deposits will be removed at that time. Samples of the deposited plasmids will be
10 replaced if the depository is unable to dispense viable samples. The invention described and claimed herein is not to be limited in scope by plasmids deposited, since the deposited embodiment is intended only as an illustration of the invention. Any equivalent or
15 similar plasmids that encode similar or equivalent antigens as described in this application are within the scope of the invention.

	<u>Plasmid</u>	<u>ATCC Designation</u>	<u>Date Deposited</u>
	PXL5	209143	July 16, 1997
20	PXL6	209144	July 16, 1997

EXAMPLES

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific
25 Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although
30 specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitations.

Methods of molecular genetics, protein biochemistry, and immunology used but not explicitly
35 described in this disclosure and these Examples are

amply reported in the scientific literature and are well within the ability of those skilled in the art.

Example 1

This Example describes the construction of vectors 5 containing the RSV G gene.

Figure 1 shows a restriction map of the gene encoding the G protein of respiratory syncytial virus and Figure 2 shows the nucleotide sequence of the gene encoding the full-length RSV G protein (SEQ ID No: 1) 10 and the deduced amino acid sequence (SEQ ID No: 2). Figure 3 shows the gene encoding the secreted RSV G protein (SEQ ID No: 3) and the deduced amino acid sequence (SEQ ID No: 4).

Plasmid pXL5 (Figure 4) was prepared for the 15 expression of the full-length RSV G protein as follows:

A recombinant Bluescript plasmid (RSV G12) containing the cDNA encoding the full-length G protein of a clinical RSV isolate (subgroup A) was used to construct vectors for RSV DNA-G immunization. RSV G12 20 was digested with AflIII and EcoRI and filled-in with the Klenow subunit of DNA polymerase. The resulting 1.23 kb fragment containing the coding sequence for the full-length G protein was gel-purified and ligated to VR-1012 (Vical) (Figure 6) previously linearized with 25 EcoRV. This procedure placed the RSV G cDNA downstream of the immediate-early cytomegalovirus (CMV) promoter and Intron A sequences of human cytomegalovirus (CMV) and upstream of the bovine growth hormone (BGH) poly-A site. The junctions of the cDNA fragments in the plasmid 30 construct were confirmed by sequencing analysis. The resulting plasmid was designated pXL5.

Plasmid pXL6 (Figure 5) was prepared for the expression of a secretory RSV G protein as follows:

RSV G12 was digested with EcoRI, filled-in with 35 Klenow and digested again with BamHI. The BamHI

cleavage resulted in the generation of a cDNA fragment encoding a RSV G protein with N-terminal truncation. This DNA segment was gel-purified and ligated in the presence of a pair of 11 mer oligodeoxynucleotides 5 (5'GATCCACTCAG 3') (SEQ ID no: 7)

3' GTGAGTCCTAG 5' (SEQ ID no: 8)

to VR-1020 (Vical) previously digested with BglII, filled in with Klenow, digested again with BamHI and gel-purified. This procedure placed the truncated RSV G 10 cDNA (lacking the coding region for the N-terminal 91 amino acid residues including the transmembrane domain) downstream of the immediate-early CMV promoter and Intron A sequences of human CMV and upstream of the BGH poly-A site. In addition, there was the introduction of 15 approximately 100 bp of 5' untranslated region and the coding sequence for the signal peptide of human plasminogen activator protein (Figure 7) fused in frame to the N-terminus of the RSV G protein coding sequence downstream of the CMV promoter/Intron A sequences. The 20 junctions of the cDNA fragments in the plasmid construct were confirmed by sequencing analysis. The resulting plasmid was designated pXL6.

Example 2

This Example describes the immunization of mice.
25 Mice are susceptible to infection by RSV as described in ref. 24.

Plasmid DNA was purified through double CsCl centrifugations. For intramuscular (i.m.) immunization, tibialis anterior muscles of BALB/c mice (male, 6 to 8 30 week old) (Jackson Lab., Bar Harbor, ME, USA) were bilaterally injected with 2 x 50 μ g (1 μ g/ μ L in PBS) of either pXL5, pXL6 or V-1012. Five days prior to DNA injection, the muscles were treated with 2 x 50 μ L (10 μ M in PBS) of cardiotxin (Latoxan, France) to increase DNA 35 uptake and enhance immune responses, as reported by

Davis et al (ref. 23). The animals were boosted with the same dose of plasmid DNA 6 weeks and 13 weeks later, respectively. For intradermal (i.d.) immunization, 100 μ g of the plasmid DNA (2 μ g/ μ L in PBS) of were 5 injected at the base of the tail and boosted 6 weeks and 13 weeks later, respectively. Mice in the positive control group were immunized intranasally (i.n.) with 10⁶ plaque forming units (pfu) of a clinical RSV strain of the A2 subtype grown in Hep2 cells kindly provided by 10 Dr. B. Graham (ref. 24).

Four weeks after the third immunization, mice were challenged intranasally with 10⁶ pfu of the RSV A2 strain. Lungs were aseptically removed 4 days later, weighed and homogenized in 2 mL of complete culture 15 medium (ref. 25). The number of pfu in lung homogenates was determined in duplicate as previously described (ref. 26) using vaccine-quality Vero cells.

Example 3

This Example describes the immunogenicity and 20 protection by polynucleotide immunization.

Antisera obtained from immunized mice were analyzed for anti-RSV G IgG antibody titres using specific enzyme-linked immunosorbent assay (ELISA) and for RSV-specific plaque-reduction titres. ELISAs were performed 25 using 96-well plates coated with immunoaffinity-purified RSV G protein (50 ng/mL) and 2-fold serial dilutions of immune sera. A goat anti-mouse IgG antibody conjugated to alkaline phosphatase (Jackson ImmunoRes., Mississauga, Ontario, Canada) was used as secondary 30 antibody. Plaque reduction titres were determined according to Prince et al (ref. 26) using vaccine-quality Vero cells. Four-fold serial dilutions of immune sera were incubated with 50 pfu of the RSV Long strain (ATCC) in culture medium at 37°C for 1 hr in the 35 presence of 5% CO₂ and the mixtures were used to infect

Vero cells. Plaques were fixed with 80% methanol and developed 5 days later using a mouse anti-RSV F monoclonal IgG1 antibody and donkey anti-mouse IgG antibody conjugated to peroxidase (Jackson ImmunoRes.,
5 Mississauga, Ontario, Canada). The RSV-specific plaque reduction titre was defined as the dilution of serum sample yielding 50% reduction in plaque number. Both ELISA and plaque reduction assays were performed in duplicate and data are expressed as the means of two
10 determinations.

The results obtained are reproduced in Tables I and II below:

Table I. Immunogenicity of DNA-G in BALB/c Mice

Immunogen Titre	Anti-RSV G IgG Titre (Log 2(titre/100))			RSV-Specific Plaque Reduction (Log 2 titre) 17 weeks
	6 weeks	10 weeks	17 weeks	
VR-1012 (i.m.)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
pXL5 (i.m.)	3.10 ± 2.77	9.70 ± 1.06	8.60 ± 1.17	5.40 ± 1.65
pXL6 (i.m.)	5.78 ± 1.20	9.30 ± 0.82	8.89 ± 1.54	7.26 ± 0.82
pXL5 (i.d.)	1.50 ± 1.27	8.60 ± 1.43	8.30 ± 1.25	7.92 ± 0.59
pXL6 (i.d.)	3.70 ± 1.25	10.30 ± 1.06	9.44 ± 1.24	6.92 ± 0.94
RSV (i.n.)	6.83 ± 0.41	9.67 ± 0.52	9.83 ± 0.41	11.80 ± 0.08

Table II. Immunoprotective Ability of DNA-G in BALB/c Mice

<i>Immunogen</i>	<i>No. Mice</i>	<i>Mean Virus Lung Titre* (pfu/g Lung) (Log 10 \pm SD)</i>	<i>No. Fully Protected Mice#</i>
VR-1012 (i.m.)	6	4.81 \pm 0.01	0
pXL5 (i.m.)	6	0.29 \pm 0.90	5
pXL6 (i.m.)	6	0.40 \pm 1.20	5
pXL5 (i.d.)	6	0.30 \pm 1.10	5
pXL6 (i.d.)	6	0.29 \pm 0.90	5
RSV (i.n.)	6	0.00 \pm 0.00	6

*Sensitivity of the assay: 10^{1.96} pfu/g lung.

The term, fully protected mice, refers to animals with no detectable RSV in the lungs 4 days post viral challenge.

As seen in Table I, plasmids pXL5 and pXL6 were found to be immunogenic following either i.m. or i.d. immunization producing anti-G antibodies and virus neutralizing antibodies. In addition, as seen in Table II, the plasmids pXL5 and pXL6 protected immunized mice against primary RSV infection of the lower respiratory tract. The control vector produced no immune response and did not confer protection.

Example 4

10 This Example describes the determination of the local lung cytokine expression profile in mice immunized with pXL5 and pXL6 after RSV challenge.

BALB/c mice were immunized at 0 and 6 weeks with 100 μ g of pXL5 and 6, prepared as described in Example 1, 15 and challenged with RSV i.n. at 10 weeks. Control animals were immunized with placebo PI-RSV and live RSV and challenged with RSV according to the same protocol. In addition, animals were immunized with pXL2, as described in copending United States Patent Application 20 no. 08/476,397 filed June 7, 1995 (WO 96/40945) and challenged with RSV, also following the same protocol. Four days post viral challenge, lungs were removed from immunized mice and immediately frozen in liquid nitrogen. Total RNA was prepared from lungs homogenized 25 in TRIzol/ β -mercaptoethanol by chloroform extraction and isopropanol precipitation. Reverse transcriptase-polymerase chain reaction (RT-PCR) was then carried out on the RNA samples using either IL-4, IL-5 or IFN- γ specific primers from CloneTech. The amplified products 30 were then liquid-hybridized to cytokine-specific 32 P-labeled probes from CloneTech, resolved on 5% polyacrylamide gels and quantitated by scanning of the radioactive signals in the gels. Three mouse lungs were removed from each treatment group and analyzed for lung 35 cytokine expression for a minimum of two times. The

data is presented in Figure 8 and represents the means and standard deviations of these determinations.

As may be seen from the data presented in Figure 8:

- 5 1. Immunization with live RSV intranasally (i.n.) resulted in a balanced cytokine profile (IFN- γ , IL-4 and IL-5), whereas that with FI-RSV intramuscularly (i.m.) resulted in a Th2 predominance (elevated IL-4 and IL-5). These results are similar to those reported in the literature.
- 10 2. Immunization with pXL5 or pXL6 via either the i.m. or intradermal (i.d.) route gave rise to a balanced cytokine profile similar to that with live RSV immunization.
- 15 3. The magnitude of the cytokine responses with i.m. pXL6 (RSV G) and pXL2 (RSV F) immunization using the construct expressing a secretory form of the protein (SEC) is significantly higher than that with live RSV immunization.
- 20 4. The magnitude of the cytokine response with pXL5 immunization using constructs expressing a full-length membrane-associated RSV G protein (MA) and i.d. pXL6 was somewhat higher than that with live RSV immunization.
- 25 5. The balanced local cytokine response observed with DNA-G immunization contrasts with that reported by Openshaw et al (ref. 13). Using a recombinant vaccinia virus expressing the G protein, these investigators reported a local Th2 response by analysis of bronchoalveolar lavage. The results herein, which were obtained through a monogenic approach, indicate that the Th2 response is not necessarily an intrinsic property of the G protein.

SUMMARY OF THE DISCLOSURE

In summary of this disclosure, the present invention provides certain novel non-replicating vectors containing genes encoding RSV G proteins, methods of immunization using such vectors and methods of diagnosis using such vectors. Modifications are possible within the scope of this invention.

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CLAIMS

What we claim is:

1. An immunogenic composition for *in vivo* administration to a host for the generation in the host of protective antibodies to respiratory syncytial virus (RSV) G protein, comprising a non-replicating vector comprising:

a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and

a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein *in vivo* from said vector in the host, and

a pharmaceutically-acceptable carrier therefor.

2. The composition of claim 1 wherein said first nucleotide sequence encodes a full-length RSV G protein.

3. The composition of claim 2 wherein said nucleotide sequence comprises the nucleotide sequence shown in Figure 2 (SEQ ID NO:1).

4. The composition of claim 2 wherein said first nucleotide sequence comprises the nucleotide sequence encoding a full length RSV G protein having the amino acid sequence shown in Figure 2 (SEQ ID NO:2).

5. The composition of claim 1 wherein said first nucleotide sequence encodes a RSV G protein from which the transmembrane coding sequence and sequences upstream thereto are absent.

6. The composition of claim 5 wherein said non-replicating vector further comprises a heterologous signal peptide encoding nucleotide sequence immediately upstream of the 5'-terminus of said first nucleotide

sequence.

7. The composition of claim 6 wherein said signal peptide encoding sequence encodes the signal peptide for human tissue plasminogen activator.
8. The composition of claim 5 wherein said first nucleotide sequence comprises the nucleotide sequence shown in Figure 3 (SEQ ID NO:3).
9. The composition of claim 5 wherein said first nucleotide sequence comprises a nucleotide sequence encoding a truncated RSV G protein having the amino acid sequence shown in Figure 3 (SEQ ID NO:4).
10. The composition of claim 1 wherein said promoter sequence is a immediate early cytomegalovirus promoter.
11. The composition of claim 1 wherein said second nucleotide sequence is the human cytomegalovirus Intron A.
12. The composition of claim 1 wherein the non-replicating vector is a plasmid vector.
13. The composition of claim 12 wherein the plasmid vector is pXL5 as shown in Figure 4.
14. The composition of claim 12 wherein the plasmid vector is pXL6 as shown in Figure 5.
15. A method of immunizing a host against disease caused by infection with respiratory syncytial virus (RSV), which comprises administering to said host an effective amount of a non-replicating vector comprising:
 - a first nucleotide sequence encoding an RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,
 - a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and
 - a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein *in vivo* from

said vector in the host.

16. The method of claim 15 wherein said first nucleotide sequence encodes a full-length RSV G protein.

17. The method of claim 16 wherein said nucleotide sequence comprises the nucleotide sequence shown in Figure 2 (SEQ ID NO:1).

18. The method of claim 16 wherein said first nucleotide sequence comprises the nucleotide sequence encoding a full length RSV G protein shown in Figure 2 (SEQ ID NO:2).

19. The method of claim 14 wherein said first nucleotide sequence encodes an RSV G protein from which the transmembrane coding sequence and sequences upstream thereto are absent.

20. The method of claim 19 wherein said non-replicating vector further comprises a heterologous signal peptide encoding nucleotide sequences immediately upstream of the 5'-terminus of said first nucleotide sequence.

21. The method of claim 20 wherein said signal peptide encoding sequence encodes the signal peptide for human tissue plasminogen activator.

22. The method of claim 19 wherein said first nucleotide sequence comprises the nucleotide sequence shown in Figure 3 (SEQ ID NO:3).

23. The method of claim 19 wherein said first nucleotide sequence comprises a nucleotide sequence encoding a transverse RSV G protein shown in Figure 3 (SEQ ID NO:4).

24. The method of claim 15 wherein said promoter sequence is an immediate early cytomegalovirus promoter.

25. The method of claim 15 wherein said second nucleotide sequence is the human cytomegalovirus Intron A.

26. The method of claim 1 wherein the non-replicating vector is a plasmid vector.

27. The method of claim 26 wherein said plasmid vector is pXL5 as shown in Figure 4.
28. The method of claim 26 wherein said vector is pXL6 as shown in Figure 5.
29. The method of claim 15 wherein a balanced Th1/Th2 immune response is induced.
30. A method of using a gene encoding a respiratory syncytial virus (RSV) G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein, to produce an immune response in a host, which comprises:
 - isolating said gene,
 - operatively linking said gene to at least one control sequence to produce a non-replicating vector, said control sequence directing expression of said RSV G protein when introduced into a host to produce an immune response to said RSV G protein, and
 - introducing said vector into a host.
31. The method of claim 30 wherein said gene encoding an RSV G protein encodes a full length RSV G protein.
32. The method of claim 30 wherein said gene encoding an RSV G protein encodes an RSV G protein lacking the transmembrane domain and sequences upstream thereto.
33. The method of claim 32 wherein said vector further comprises a signal peptide encoding nucleotide sequences immediately upstream of the 5'-terminus of said first nucleotide sequence.
34. The method of claim 33 wherein said signal peptide encoding sequence encodes the signal peptide for human tissue plasminogen activator.
35. The method of claim 30 wherein said at least one control sequence comprises the immediate early cytomegalovirus promoter.
36. The method of claim 35 including the step of:
operatively linking said gene to an

immunoprotection enhancing sequence to produce an enhanced immunoprotection to said RSV G protein in said host.

37. The method of claim 36 wherein said immunoprotection enhancing sequence is introduced into said vector between said control sequence and said gene.

38. The method of claim 37 wherein said immunoprotection enhancing sequence is the human cytomegalovirus Intron A.

39. The method of claim 30 wherein said gene is contained within a plasmid selected from the group consisting of pXL5 and pXL6.

40. A method of producing a vaccine for protection of a host against disease caused by infection with respiratory syncytial virus (RSV), which comprises:

isолating a first nucleotide sequence encoding an RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

operatively linking said first nucleotide sequence to at least one control sequence to produce a non-replicating vector, the control sequence directing expression of said RSV G protein when introduced to a host to produce an immune response to said RSV G protein,

operatively linking said first nucleotide sequence to a second nucleotide sequence to increase expression of said RSV G protein *in vivo* from the vector in the host, and

formulating said vector as a vaccine for *in vivo* administration to a host.

41. The method of claim 40 wherein said vector is selected from group consisting of pXL5 and pXL6.

42. A vaccine produced by the method of claim 40.

43. A method of determining the presence of a respiratory syncytial virus (RSV) G protein in a sample,

comprising the steps of:

- (a) immunizing a host with a non-replicating vector to produce antibodies specific for the RSV G protein, said non-replicating vector comprising:
 - a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,
 - a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and
 - a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein *in vivo* from said vector in the host,
- (b) isolating the RSV G protein specific antibodies;
- (c) contacting the sample with the isolated antibodies to produce complexes comprising any RSV G protein present in a sample and said isolated RSV G protein-specific antibodies; and
- (d) determining the production of the complexes.

44. The method of claim 43 wherein said vector is selected from the group consisting of pXL5 and pXL6.

45. A diagnostic kit for detecting the presence of a respiratory syncytial virus (RSV) G protein in a sample, comprising:

- (a) a non-replicating vector capable of generating antibodies specific for the RSV G protein when administered to a host, the non-replicating vector comprising:
 - a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,
 - a promoter sequence operatively coupled to

said first nucleotide sequence for expression of said RSV G protein in the host, and

a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein *in vivo* from said vector in the host;

(b) isolation means to isolate said RSV G protein-specific antibodies;

(c) contacting means to contact the isolated RSV G specific antibodies with the sample to produce a complex comprising any RSV G protein in the sample and RSV G protein specific antibodies, and

(d) identifying to determine production of the complex.

46. The diagnostic kit of claim 45 wherein said vector is selected from the group consisting of pXL5 and pXL6.

47. A method for producing antibodies specific for a G protein of respiratory syncytial virus (RSV) comprising:

(a) immunizing a host with an effective amount of a non-replicating vector to produce RSV G-specific antibodies, said non-replicating vector comprising:

a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and

a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein *in vivo* from said vector in the host; and

(b) isolating the RSV G-specific antibodies from the host.

48. A method of producing monoclonal antibodies specific for a G protein of respiratory syncytial virus (RSV) comprising the steps of:

(a) constructing a vector comprising:

a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and

a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein *in vivo* from said vector in the host;

(b) administering the vector to at least one mouse to produce at least one immunized mouse;

(c) removing B-lymphocytes from the at least one immunized mouse;

(d) fusing the B-lymphocytes from the at least one immunized mouse with myeloma cells, thereby producing hybridomas;

(e) cloning the hybridomas;

(f) selecting clones which produce anti-RSV G protein antibody;

(g) culturing the anti-RSV G protein antibody-producing clones; and then

(h) isolating anti-RSV G protein antibodies from the cultures.

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Restriction Map of the RSV G Gene

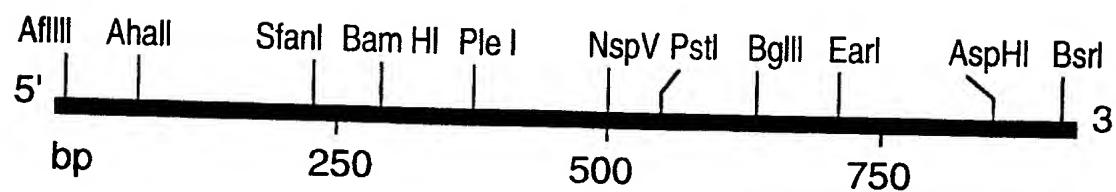


FIG.1

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FIG.2A

10	19	28	37	46	55
TGCCAAC ATG TCC AAA AAC AAG GAC CAA CGC ACC GCT AAG ACA CTA GAA AAG ACC					
Met Ser Lys Asn Lys Asp Gln Arg Thr Ala Lys Thr Leu Glu Lys Thr					
64	73	82	91	100	109
TGG GAC ACT CTC AAT CAT TTA TTA TTC ATA TCA TCG GGC TTA TAT AAG TTA ATT					
Trp Asp Thr Leu Asn His Leu Leu Phe Ile Ser Ser Gly Leu Tyr Lys Leu Asn					
118	127	136	145	154	163
CTT AAA TCT GTA GCA CAA ATC ACA TTA TCC ATT CTG GCA ATT ATC TCA ACT					
Leu Lys Ser Val Ala Gln Ile Thr Leu Ser Ile Leu Ala Met Ile Ile Ser Thr					
172	181	190	199	208	217
TCA CTT ATA ATT ACA GCC ATC ATA TTC ATA GCC TCG GCA AAC CAC AAA GTC ACA					
Ser Leu Ile Ile Thr Ala Ile Ile Phe Ile Ala Ser Ala Asn His Lys Val Thr					
226	235	244	253	262	271
CIA ACA ACT GCA ATC ATA CAA GAT GCA ACA AGC CAG ATC AAG AAC ACA ACC CCA					
Leu Thr Thr Ala Ile Ile Gln Asp Ala Thr Ser Gln Ile Lys Asn Thr Thr Pro					
280	289	298	307	316	325
ACA TAC CTC ACT CAG GAT CCT CAG CTT GGA ATC AGC TTC TCC ATT CTG TCT GAA					
Thr Tyr Leu Thr Gln Asp Pro Gln Leu Gly Ile Ser Phe Ser Asn Leu Ser Glu					

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FIG.2B

334	343	352	361	370	379
ATT ACA TCA CAA ACC ACC ACC ATA CTA GCT TCA ACA ACA CCA GGA GTC AAG TCA					
Ile Thr Ser Gln Thr Thr Ile Leu Ala Ser Thr Thr Pro Gly Val Lys Ser					
388	397	406	415	424	433
AAC CTG CAA CCC ACA ACA GTC AAG ACT AAA AAC ACA ACA ACC CAA ACA CAA					
Asn Leu Gln Pro Thr Thr Val Lys Thr Lys Asn Thr Thr Thr Gln Thr Gln					
442	451	460	469	478	487
CCC AGC AAG CCC ACT ACA AAA CAA CGC CAA AAC AAA CCA CCA AAC AAA CCC AAT					
Pro Ser Lys Pro Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro Asn Lys Pro Asn					
496	505	514	523	532	541
AAT GAT TTT CAC TTC GAA GIGC TTT AAC TTT GTA CCC TGC ACC ATA TGC AGC AAC					
Asn Asp His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys Ser Asn					
550	559	568	577	586	595
AAT CCA ACC TGC TGG GCT ATC TGC AAA AGA ATA CCA AAC AAA AAA CCA GGA AAG					
Asn Pro Thr Cys Trp Ala Ile Cys Lys Arg Ile Pro Asn Lys Lys Pro Gly Lys					
604	613	622	631	640	649
AAA ACC ACC ACC AAG CCT ACA AAA AAA CCA ACC TTC AAG ACA ACC AAA AAA GAT					
Lys Thr Thr Lys Pro Thr Lys Pro Thr Phe Lys Thr Thr Lys Lys Asp					

FIG.2C

658	667	676	685	694	703
CTC AAA CCT CAA ACC ACT AAA CCA AAG GAA GTT CCC ACC ACC AAG CCC ACA GAA					
Ieu Lys Pro Gln Thr Thr Lys Pro Lys Glu Val Pro Thr Thr Lys Pro Thr Glu					
712	721	730	739	748	757
GAG CCA ACC ATC ACC AAA ACA AAC ATC ACA ACT ACA CTG CTC ACC AAC					
Glu Pro Thr Ile Asn Thr Thr Lys Thr Asn Ile Thr Thr Thr Leu Leu Thr Asn					
766	775	784	793	802	811
AAC ACC ACA GGA AAT CCA AAA CTC ACA AGT CAA ATG GAA ACC TTC CAC TCA ACC					
Asn Thr Thr Gly Asn Pro Lys Leu Thr Ser Gln Met Glu Thr Phe His Ser Thr					
820	829	838	847	856	865
TCC TCC GAA GGC AAT CTA AGC CCT TCT CAA GTC TCC ACA ACA TCC GAG CAC CCA					
Ser Ser Glu Gly Asn Leu Ser Pro Ser Gln Val Ser Thr Thr Ser Glu His Pro					
874	883	892	901	914	
TCA CAA CCC TCA TCT CCA CCC AAC ACA ACA CGC CAG TAGTTATTAA AAAAANAA					
Ser Gln Pro Ser Ser Pro Pro Asn Thr Thr Arg Gln					

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FIG.3A

CAC AAA GTC ACA CTA ACA ACT GCA ATC ATA CAA GAT GCA ACA AGC CAG CAG AAG	54
His Lys Val Thr Leu Thr Thr Ala Ile Ile Gln Asp Ala Thr Ser Gln Ile Lys	18
AAC ACA ACC CCA ACA TAC CTC ACT CAG GAT CCT CAG CTT GGA ATC AGC TTC TCC	108
Asn Thr Pro Thr Tyr Leu Thr Gln Asp Pro Gln Leu Gly Ile Ser Phe Ser	36
AAT CTG TCT GAA ATT ACA TCA CAA ACC ACC ATA CTA GCT TCA ACA ACA CCA	162
Asn Leu Ser Glu Ile Thr Ser Gln Thr Thr Ile Leu Ala Ser Thr Thr Pro	54
GCA GTC AAG TCA AAC CTG CAA CCC ACA ACA GTC AAG ACT AAA AAC ACA ACA	216
Gly Val Lys Ser Asn Leu Gln Pro Thr Thr Val Lys Thr Asn Thr Thr Thr	72
ACC CAA ACA CAA CCC AGC AAG CCC ACT ACA AAA CAA CCG CAA AAC AAA CCA CCA	270
Thr Gln Thr Gln Pro Ser Lys Pro Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro	90
AAC AAA CCC AAT AAT GAT TTT CAC TTC GAA GTG TTT AAC TTT GIA CCC TGC AGC	324
Asn Lys Pro Asn Asn Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Cys Ser	108
ATA TGC AGC AAC AAT CCA ACC TGC TGG GCT ATC TGC AAA AGA ATA CCA AAC AAA	378
Ile Cys Ser Asn Asn Pro Thr Cys Trip Ala Ile Cys Lys Arg Ile Pro Asn Lys	126
AAA CCA CGA AAG AAA ACC ACC AAG CCT ACA AAA AAA CCA ACC TTC AAG ACA	432
Lys Pro Gly Lys Lys Lys Pro Thr Thr Lys Pro Thr Phe Lys Thr	144

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FIG.3B

ACC AAA AAA GAT CTC AAA CCT CAA ACC ACT AAA CCA AAG GAA GTC CCC ACC ACC	486
Thr Lys Lys Asp Leu Lys Pro Gln Thr Thr Lys Pro Lys Glu Val Pro Thr Thr Thr	162
AAG CCC ACA GAA GAG CCA ACC ATC AAC ACC ACC AAA ACA AAC ATC ACA ACT ACA	540
Lys Pro Thr Glu Glu Pro Thr Ile Asn Thr Thr Asn Thr Lys Thr Asn Ile Thr Thr Thr	180
CTG CTC ACC AAC ACC ACA GGA AAT CCA AAA CTC ACA AGT CAA ATG GAA ACC	594
Leu Leu Thr Asn Asn Thr Thr Gly Asn Pro Lys Leu Thr Ser Gln Met Glu Thr	198
TTC CAC TCA ACC TCC TCC GAA CGC AAT CTA AGC CCT CCT CAA GTC TCC ACA ACA	648
Phe His Ser Thr Ser Ser Glu Gly Asn Leu Ser Pro Ser Gln Val Ser Thr Thr	216
6/19	
TCC GAG CAC CCA TCA CAA CCC TCA TCT CCA CCC AAC ACA ACA CGC CAG TAG	699
Ser Glu His Pro Ser Gln Pro Ser Pro Pro Asn Thr Arg Gln	232

TTATTA AAAAAAAA

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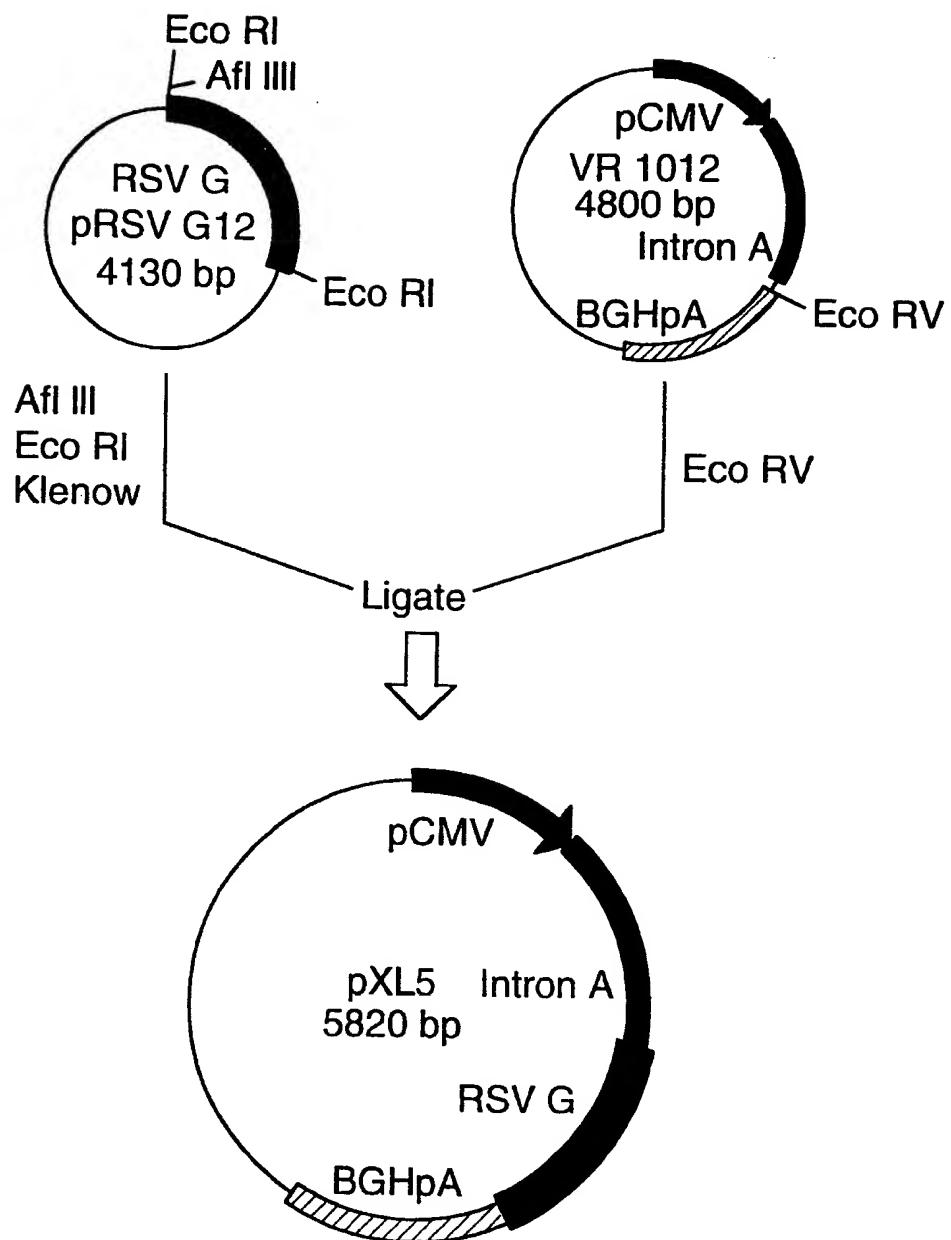


FIG.4.

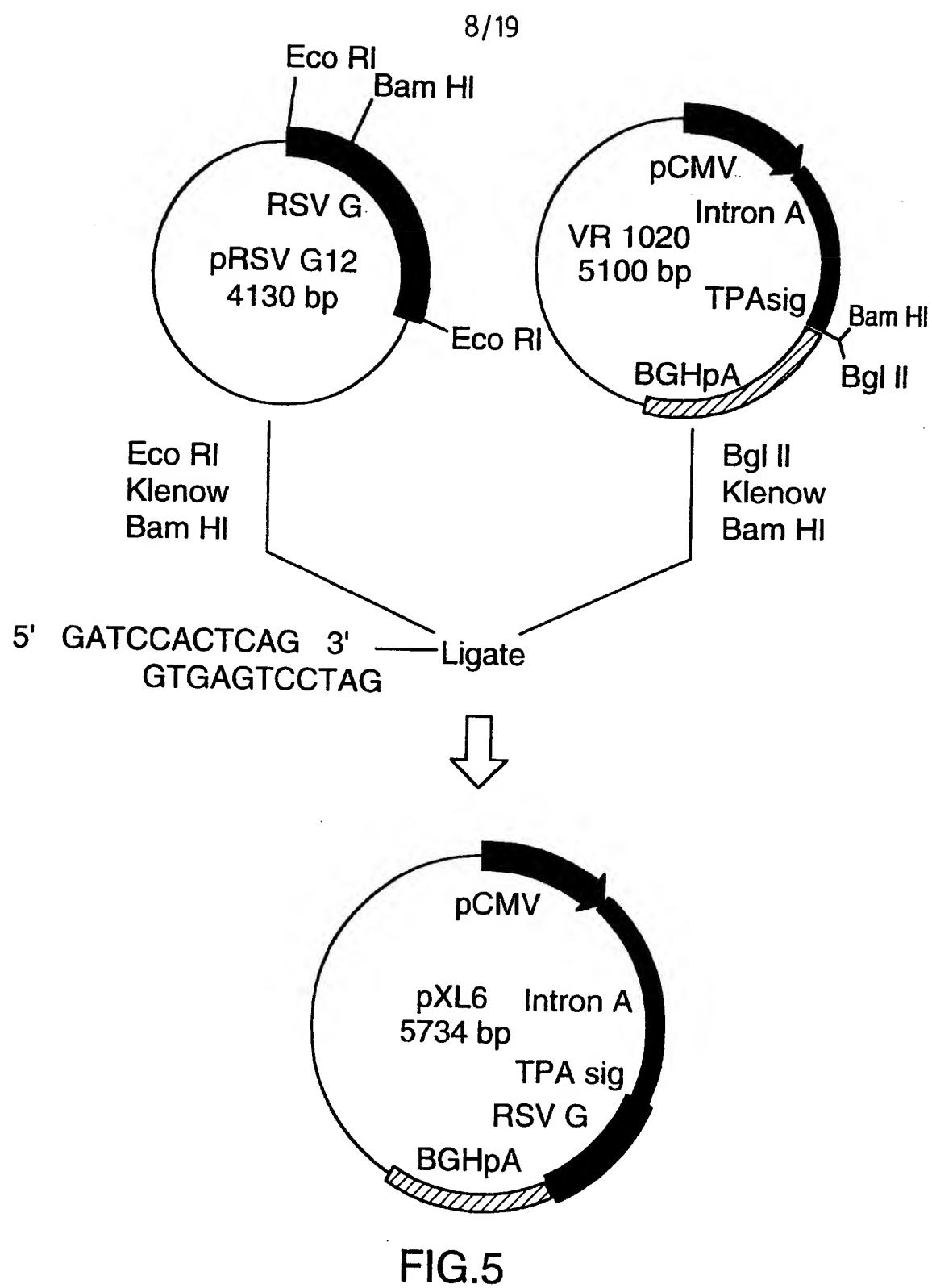


FIG. 6A

10 20 30 40 50 60 70
 TCCCGGTTT CCGTCATGAC GGTGAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA CAGCTTGCT
 80 90 100 110 120 130 140
 GTTAAGCCGAT GCGGGGAGCA GACAAGCCCG TCAGGGCGC TAGGCCGGTG TTGCCCCGGTG TCGGGCTGG
 150 160 170 180 190 200 210
 CTAAACTATG CGGCGATCAGA GGAGATTTGA CTGAGAGTCG ACCATAATGCG GTGGAATA CGGCACAGAT
 220 230 240 250 260 270 280
 GCGTAAGGAG AAAATTACCGC ATCAGATTGG CTATTGGCA TTGCTAACGT TGTATCCATA TCATAATATG
 290 300 310 320 330 340 350
 TACATTATA TTGGCTCATG TCCAACATTA CGGCCATGTT GACATTGATT ATTGACTAGT TATTAAATGT
 360 370 380 390 400 410 420
 AATCAATTAC GGGGTCAATTAA GTTCATAGCC CTAATATGCA GTTCCGGGT ACATAACTTA CGGTAAATGG
 430 440 450 460 470 480 490
 CCCGCTGGC TGACCCGCCA AGGACCCCG CCATTGAGC TCAATTAATCA CGTAATGTTCC CATACTAACG
 500 510 520 530 540 550 560
 CCAAATAGGA CTTCATGG ACCTCAATTG GTGGGATTAAT TACGGTAAAC TGCCCACTTG GCAGTACATC

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FIG. 6B

570 580 590 600 610 620 630
 AAGTGTATCA TATGCCAAGT ACCGCCCCTA TTGACCTCAA TGACCGTAAA TGCCCCGGCT GGCATTATGC
 640 650 660 670 680 690 700
 CCAGTACATG ACCTTATGGG ACTTTCTTAC TTGGCAGTAC ATCTAACAT TACTCATCC TATTACCATG
 710 720 730 740 750 760 770
 GTGATGGGT TTTCGCGTA CATCAATGGG CGTGGATAGC GGTTCGACTC ACCTGGATTC CCAAGTCTCC
 780 790 800 810 820 830 840
 ACCCCATCA CGTCAATGGG AGTTTGTTTT GGCACCAAA TCAACGGAC TTTCACAAAT GTCTAAACAA
 850 860 870 880 890 900 910
 CTCCACCCA TTGACCCAA TGGCGGTAG CGGTGTACGG TGGAGGGCT ATTATAACCG AGCTCGTTA
 920 930 940 950 960 970 980
 GIGAACCGTC AGATGGCTG GAGACGCCAT CCAGGCTGTT TTGACCTCA TAGAGACAC CGGGACCGAT
 990 1000 1010 1020 1030 1040 1050
 CCAGGCTCG CGGGCGGAA CGCTGCATTG GAAGGGGAT TCCCGTGGC AAGAGTGACG TAAGTACCGC
 1060 1070 1080 1090 1100 1110 1120
 CTATAGACTC TATAGGCACA CCCCTTGGC TCCTATGCAAT GCTATACTGT TTTCGCTTG GGGCTATAC

FIG.6C

1130 1140 1150 1160 1170 1180 1190
 ACCCCCCGTT CCTTATGCTA TAGGTGATGG TATAGCTTAG CCTATAGGTG TCGGTATTG ACCATTATTG
 1200 1210 1220 1230 1240 1250 1260
 ACCACTCCCC TATTGGTGAC GATACTTTC ATTACTAAC CATAACATGG CTCCTGCCA CAACTATCTC
 1270 1280 1290 1300 1310 1320 1330
 TATTGGCTAT ATGCCAATAC TCTGTCTTC AGAACATGAC ACCAACATCTG TATTTTACA GCATGGGTC
 1340 1350 1360 1370 1380 1390 1400 11/19
 CCATTAAATT TTTACAAATT CACATATACA ACAACGGGT CCCCGTCCC CGCAGTTTT ATTAAACATA
 1410 1420 1430 1440 1450 1460 1470
 CGGTGGATC TCCACCGMA TCTGGGTAC GTGTTCCGA CATGGCTCT TCTGGGTAG CGGGGGAGCT
 1480 1490 1500 1510 1520 1530 1540
 TCCACATCCG AGCCCTGGTC CCATGCCTCC AGGGCTCAT GGTGGCTGG CAGCTCTTG CTCTAAACAG
 1550 1560 1570 1580 1590 1600 1610
 TGGAGGCCAG ACTTACGCAC ACCACAAATGC CCACCAACC CAGTGGCG CACAAGGCCG TGGGGTAGG
 1620 1630 1640 1650 1660 1670 1680
 GTATGTTCT GAAATGAGC GTGGGACATTG GCTCGCACG GCTGAGCGCAG ATGGAAGACT TAAGGCAGCG

FIG. 6D

1690 1700 1710 1720 1730 1740 1750
 GCAGAACGAG ATGCCGGCAG CTGAGTGTGT GTATTCTGAT AAGAGTCAGA GGTAACTCCG GTTCCCGTGC

 1760 1770 1780 1790 1800 1810 1820
 TGTAAACGGT CGAGGGCACT GIAAGTCAG CAGTACTCGT TCTTCGGCG CGGGCCACCA GACATAATAG

 1830 1840 1850 1860 1870 1880 1890
 CTCAGCACT AACAGACTGT TCCCTTCAT GGGTCTTTTC TCCAGTCACC GTCTGAGACA CGTGTGATCA

 1900 1910 1920 1930 1940 1950 1960 12 / 19
 GATATGGGG CCCTCTAGA CCAGGGCGCT GGATCCAGAT CGCTGTGCC TTCTAGTTCG CAGCCATCTG

 1970 1980 1990 2000 2010 2020 2030
 TGTGTTGCC CTCCCCGGT CCTTCCTTGA CCCTGAAAGG TGCCACTCCG ACTGTCCCTT CCTAATAAA

 2040 2050 2060 2070 2080 2090 2100
 TGAGGAAATT GCATCGCATT GTCTGAGTAG GTGTCATTCT ATTCTCGCGG GTCTGGGGG GCAGGACAGC

 2110 2120 2130 2140 2150 2160 2170
 AAGGGGAGG ATTGGAAAGA CAATAGCAGG CATGCTGGG ATGGGGGGG CTCTATGGGT ACCCAGGTGC

 2180 2190 2200 2210 2220 2230 2240
 TGAAGAAATTG ACCCGGTTCC TCTTGCCCA GAAAGAAGCA GGCACATCCC CTTCCTGTG ACACACCCCTG

FIG.6E

2250 2260 2270 2280 2290 2300 2310
 TCCACCCCCC TGTTTCTTAG TTCCAGCCCC ACTCATAGGA CACTCATAGC TCAGGGGGGC TCGGCCTCA
 2320 2330 2340 2350 2360 2370 2380
 ATCCCACCG CTAAGTACT TGGAGCGTC TCTCCCTCC TCATTCAGGCC ACCAAACCAA ACCTAACCTC
 2390 2400 2410 2420 2430 2440 2450
 C2AGAGTGGG AACAAATTAA AGCAAGATAG GCTATTAAGT GCAGAGGGAG AGAAAATGCC TCCAACATGT
 2460 2470 2480 2490 2500 2510 2520
 GAGGAAGTAA TGZGAGAAT CATAGAATT CTTCGCTTC CTGCGCTACT GACTCGCTGC GCTCGGTGCT
 2530 2540 2550 2560 2570 2580 2590
 TCGCTGGG CGAGCGGTAT CAGCTCACTC AAAGGGGTAA ATACGGTTAT CCACAGGAATC AGGGGATAAAC
 2600 2610 2620 2630 2640 2650 2660
 GGAGGAAGA ACATGTGAGC AAAAGCCAG CAAAGGCCA CGAACCGTAA AAAGGCGGGG TTGCTGGCGT
 2670 2680 2690 2700 2710 2720 2730
 TTTCATAG GCTccccccc CCTCAACCAG ATCACAAAAA TCGACGCTCA AGTCAGAGGT GGCGAAACCC
 2740 2750 2760 2770 2780 2790 2800
 GACAGGACTA TAAAGATACC AGGGGTTCC CCCTGGAAAGC TCCCTGGTGC GCTCTCTACTGT TCGGACCTG

FIG. 6F

2810	2820	2830	2840	2850	2860	2870
CGGCTTACCG	GATAACCTGTC	CCCTTTCTC	CCTTCGGAA	GGTGGGGCT	TTCTCATAGC	TCAAGGTGA
2880	2890	2900	2910	2920	2930	2940
GGTATCTCAG	TTCGGTGTAG	GTCGGTTCGCT	CCAAGCTGGG	CTGTTGCAC	GAACCCCCG	TTCAGGCCA
2950	2960	2970	2980	2990	3000	3010
CGGCTGGCC	TTCATCGGTA	ACTATCGCT	TGACTTCAAAC	CCGGTAAAC	ACCAGTTAAC	CCGAACTGGCA
3020	3030	3040	3050	3060	3070	3080
GCAGCCACTG	GTAAACAGGAT	TAGCAGAGCG	AGGTATGTAG	GGGGTGTAC	AGAGTTCTTG	AAGTGGTGGC
3090	3100	3110	3120	3130	3140	3150
CTAACTACGG	CTACACTAGA	AGAACAGTAT	TTGGTATCTG	CGCTCTGCTG	AAGCCAGTAA	CCTTCGGAAA
3160	3170	3180	3190	3200	3210	3220
AAAGAGTTGGT	ACCTCTCTAT	CCGGCAAAACA	AACCAACGCT	GGTAGGGGTG	GTTTTTGT	TTCGAAGCAG
3230	3240	3250	3260	3270	3280	3290
CAGATTACGC	GCZAGAAAAA	AGGATCTCAA	GAAGATCTT	TGATCTTTC	TACGGGTCT	GACGCTCTAGT
3300	3310	3320	3330	3340	3350	3360
CGAACCGAAA	CTGACGTTAA	GGGATTTCG	TCATGAGATT	ATCAAAAGG	ATCTTCACT	AGATCCTTT

FIG.6G

3370 3380 3390 3400 3410 3420 3430
 AAATTAAAAA TGAAGTTTA AATCAATCTA AAGTATATAT GAGTAAACTT GGTCTGACAG TTACCAATGC
 3440 3450 3460 3470 3480 3490 3500
 TTATCAGTG AGGCACCTAT CTCAGGGATC TGTCTTATTC GTTCATCCAT AGTTGCCGTA CTGGGGGGG
 3510 3520 3530 3540 3550 3560 3570
 GGGGGCGCTG AGGTCTGCTT CGTCAAGAAG GGTGTTCTCA CTCATACCG GCCTGAATCG ACCCATCATC
 3580 3590 3600 3610 3620 3630 3640 15/19
 CAGGCCAGAAA GTGAGGGAGC CACGGTTGAT GAGGGCTTG TTGTACCGTGG ACCAGTTGGT GATTGGAAC
 3660 3670 3680 3690 3700 3710
 TTTTCCTTTC CCACCGAACG GCTGCGCTTG TGGGGAAAGAT GCGTGTATCTG ATCCCTAAC TCAGCAAAG
 3720 3730 3740 3750 3760 3770 3780
 TTGCAATTAT TCAACAAAGC CCCCGTCCCG TCAAGTCAGC GIAATGCTCT GCCAGTGTAA CAACCAATTA
 3790 3800 3810 3820 3830 3840 3850
 ACCAATTGTG ATTACAAAAA CTCATCGAGC ATCAAAATGAA ACTGCAATT ATTCAATCA GGATTATCAA
 3860 3870 3880 3890 3900 3910 3920
 TACCATATT TTCAAAAGC CGTTCTGTAA ATGAAGGAGA AAACTCAACCG AGGCAGTTCG ATAGGATGCG

FIG. 6H

3930 3940 3950 3960 3970 3980 3990
 AATATCCTGG TATCGGTCTG CGATTCCGAC TCGTCCAACA TCAATAAAC CTATTAATTTC CCCCTCTGGTCA
 4000 4010 4020 4030 4040 4050 4060
 AAAATAAGGT TATCAAGTGA GAAATCACCA TGAGTGACCA CTGAAATCCGG TCAGAATGGC AAAACCTTAT
 4070 4080 4090 4100 4110 4120 4130
 GCAATTCTTT CCAGACTTGT TCAACAGGCC ACCCAATTAGG CTCGTCATCA AAATCACTGG CATCAACCAA
 4140 4150 4160 4170 4180 4190 4200 ¹⁵
 ACCGTTATTIC ATTGTTGATT GCGGCTGAGC GAGACCAAAT ACCGGATCGC TGTTAAAGG ACAATTACAA ¹⁹
 4210 4220 4230 4240 4250 4260 4270
 ACAGGAATCG AATGCAAACG GCGCAGGAAC ACTGCGAGGG CATAACAAT ATTTCACCT GAATCZAGGAT
 4280 4290 4300 4310 4320 4330 4340
 ATTCTTCTAA TACCTGGAT CCTGTTTCG CGGGGATCGC AGTGGTCACT AACCATGGCAT CATCAAGGAT
 4350 4360 4370 4380 4390 4400 4410
 AGGGATAAA TGCCTGATGG TOGGAAGAGG CATAAAATTCC GTCAAGCCAGT TTAGTCTGAC CATCTCATCT
 4420 4430 4440 4450 4460 4470 4480
 GTAACATCAT TGCGAACTGCT ACCTTTGGCA TGTITCAAA ACAACCTCTGG CGGATTCGGGC TTCCCATACA

FIG. 6I

4490 4500 4510 4520 4530 4540 4550
 ATCGATAGAT TGTGCGACT GATTGCCGA CATTATUGCG AGCCCATTTA TACCCATAAA AATCAGGCATC
 4560 4570 4580 4590 4600 4610 4620
 CATGTTGAA TTAAATCGCG GCCTGGAGCA AGACGTTTCC CGTTCAATAT GGCTCATAAC GTTCCTTGTA
 4630 4640 4650 4660 4670 4680 4690
 TTACTGTTA TGTAAGCAGA CAGTTTATT GTTCATGATG ATATATTTT ATCTTGTGCA ATGTAACATC
 4700 4710 4720 4730 4740 4750 4760 4770
 AGAGGATTG AGACACAACG TGCCTTCCC CCCCCCCCA TTATTGAGC ATTATCAGG GTTATTCGCT
 CATGAGCGGA TACATATTG AATGTATTAA GAAAATAAA CAAATAGGGG TTCCCGGCCAC ATTTCGGGA
 4840 4850 4860 4870 4880 4890 4900
 AAAGTGCAC CTGAGCTTA AGAACCAT ATTATCATGA CATTACCTA TAAATAGG CGTATCAGGA
 4910
 GCCCCTTTGG TC

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FIG.7

10 20 30 40 50 60 70
CTGCAGTCAC CGTUGTGGAC CAGAGCTGAG ATGCCAACAGG AGTCAGGGC TGGACGAAA ACCTCTGGCA
80 90 100 110 120 130 140
GAAAGCGAA GGAGCCAGGCC GTGAATTTAA GGACCGCTGT GAAGCAATCA TGGATGCCAT GAAAGCAGGG
150 160 170 180
CTCTGCTGAG TTCTGCTGCT GTGTCGAGCA GTCTTCGTT CGCCCCAGC

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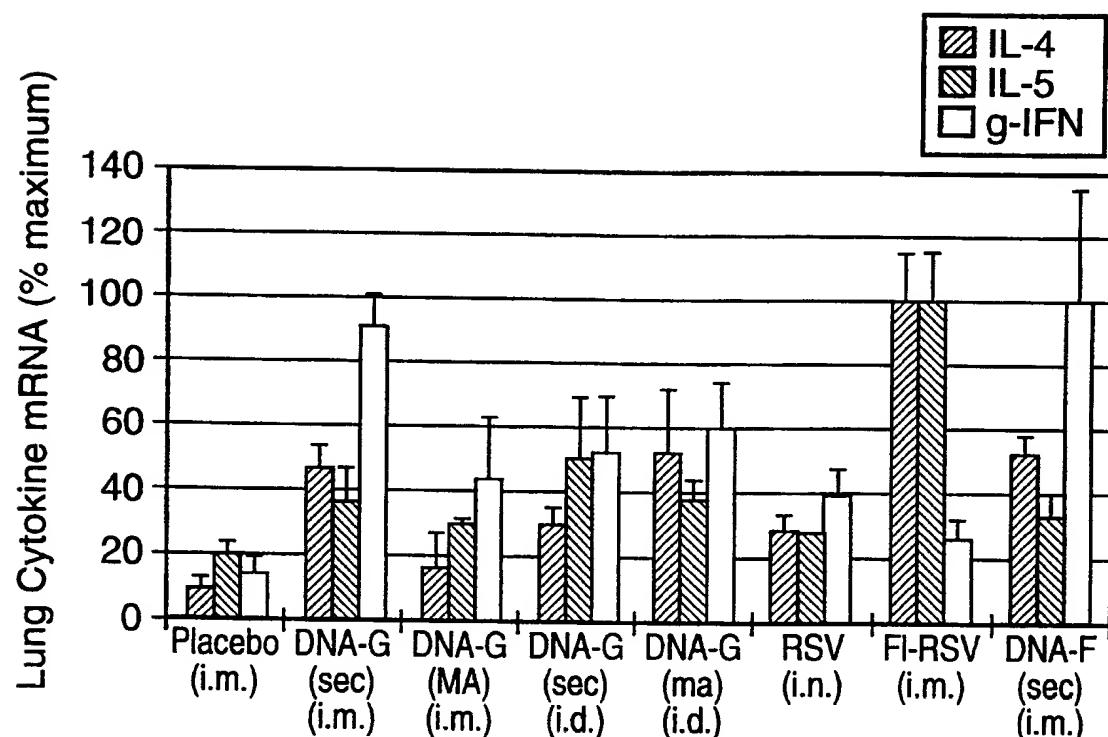


FIG.8

INTERNATIONAL SEARCH REPORT

Intern	al Application No
PCT/CA 98/00697	

A. CLASSIFICATION OF SUBJECT MATTER				
IPC 6	C12N15/45	A61K48/00	G01N33/53	C07K16/10

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>STOTT E J ET AL: "Human respiratory syncytial virus glycoprotein G expressed from a recombinant vaccinia virus vector protects mice against live-virus challenge."</p> <p>JOURNAL OF VIROLOGY, (1986 NOV) 60 (2) 607-13. JOURNAL CODE: KCV. ISSN: 0022-538X., XP002080963</p> <p>United States</p> <p>see page 607</p> <p>see abstract</p> <p>see page 608 'Results', first paragraph</p> <p>see page 609; figure 1</p> <p>---</p> <p style="text-align: center;">-/--</p>	30-35

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "Z" document member of the same patent family

Date of the actual completion of the international search

15 October 1998

Date of mailing of the international search report

30/10/1998

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Authorized officer

Sitch, W

INTERNATIONAL SEARCH REPORT

Intern	al Application No
PCT/CA 98/00697	

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>HSU K H ET AL: "Immunogenicity of recombinant adenovirus- respiratory syncytial virus vaccines with adenovirus types 4, 5, and 7 vectors in dogs and a chimpanzee."</p> <p>JOURNAL OF INFECTIOUS DISEASES, (1992 OCT) 166 (4) 769-75. JOURNAL CODE: IH3. ISSN: 0022-1899., XP002080964</p> <p>United States see page 769 see abstract</p> <p>---</p>	30-35
X	<p>STOTT E J ET AL: "Immune and histopathological responses in animals vaccinated with recombinant vaccinia viruses that express individual genes of human respiratory syncytial virus."</p> <p>JOURNAL OF VIROLOGY, (1987 DEC) 61 (12) 3855-61. JOURNAL CODE: KCV. ISSN: 0022-538X., XP002080965</p> <p>United States see page 3855 see abstract see page 3856, 'Materials and Methods', first and second paragraphs</p> <p>---</p>	30-35
X	<p>ELANGO N ET AL: "Resistance to human respiratory syncytial virus (RSV) infection induced by immunization of cotton rats with a recombinant vaccinia virus expressing the RSV G glycoprotein."</p> <p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1986 MAR) 83 (6) 1906-10. JOURNAL CODE: PV3. ISSN: 0027-8424., XP002080966</p> <p>United States see page 1906 see abstract see page 1907; figure 1</p> <p>---</p>	30-35
A	<p>ROBERTS S R ET AL: "The membrane-associated and secreted forms of the respiratory syncytial virus attachment glycoprotein G are synthesized from alternative initiation codons."</p> <p>JOURNAL OF VIROLOGY, (1994 JUL) 68 (7) 4538-46. JOURNAL CODE: KCV. ISSN: 0022-538X., XP002080967</p> <p>United States see page 4538 see abstract see page 4541; figure 1</p> <p>---</p>	5,6,8,9, 19,20, 22,23, 28,32,33

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INTERNATIONAL SEARCH REPORT

Internat	Application No
	PCT/CA 98/00697

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>NGUYEN T N ET AL: "Hydrophobicity engineering to facilitate surface display of heterologous gene products on <i>Staphylococcus xylosus</i>." JOURNAL OF BIOTECHNOLOGY, (1995 OCT 16) 42 (3) 207-19. JOURNAL CODE: AL6. ISSN: 0168-1656., XP000561819 Netherlands see page 207 see abstract</p> <p>---</p>	5,6,8,9, 19,20, 22,23, 28,32,33
A	<p>WATHEN M W ET AL: "Characterization of a novel human respiratory syncytial virus chimeric FG glycoprotein expressed using a baculovirus vector." JOURNAL OF GENERAL VIROLOGY, (1989 OCT) 70 (PT 10) 2625-35. JOURNAL CODE: I9B. ISSN: 0022-1317., XP002080968 ENGLAND: United Kingdom see page 2625 see abstract</p> <p>---</p>	5,6,8,9, 19,20, 22,23, 28,32,33
A	<p>US 5 620 896 A (HERRMANN JOHN E ET AL) 15 April 1997 see column 2, line 62 - column 5, line 31; figures 1A,1B,3,4A,4B</p> <p>---</p>	1-29, 36-48
P,X	<p>SCHRIJVER R S ET AL: "Comparison of DNA application methods to reduce BRSV shedding in cattle" VACCINE, vol. 16, no. 2-3, 2 January 1998, page 130-134 XP004098613 see the whole document</p> <p>---</p>	1-48
P,X	<p>JOHNSON T R ET AL: "Priming with secreted glycoprotein G of respiratory syncytial virus (RSV) augments interleukin-5 production and tissue eosinophilia after RSV challenge." JOURNAL OF VIROLOGY, (1998 APR) 72 (4) 2871-80. JOURNAL CODE: KCV. ISSN: 0022-538X., XP002080969 United States see page 2871 see abstract</p> <p>---</p>	30-35

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA 98/00697

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:

because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 15-39 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. Claims Nos.:

because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. Claims Nos.:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internal Application No
PCT/CA 98/00697

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5620896	A 15-04-1997	US 5643578 A	01-07-1997
		AU 3150295 A	07-11-1996
		EP 0821583 A	04-02-1998
		WO 9632932 A	24-10-1996
		CA 2181832 A	03-08-1995
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